

Reduced Responsiveness to Endothelin-1 in Peripheral Resistance Vessels of Patients With Syndrome X

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Objectives. This study sought to assess the contribution and action of nitric oxide and endothelin-1 in peripheral resistance vessels of patients with syndrome X.

Background. Patients with syndrome X may have a generalized disorder of vascular and endothelial function, promoting vasospasm.

Methods. Changes in blood flow responses to intrabrachial infusion of the endothelium-dependent vasodilators substance P and acetylcholine, the endothelium-independent nitric oxide donor sodium nitroprusside and the endothelin type A (ET_A) receptor antagonist BQ-123 were assessed using venous occlusion plethysmography in 10 patients with syndrome X and 10 matched control subjects. Vasoconstrictor responses to the nitric oxide synthase inhibitor L-N^G-monomethyl arginine (L-NMMA) and endothelin-1 were also determined.

Results. There were no significant differences in the responses to acetylcholine, substance P, sodium nitroprusside or BQ-123 between patients and control subjects. However, despite similar

degrees of vasoconstriction in response to L-NMMA in both groups, endothelin-1 caused a reduction in forearm blood flow of only $20 \pm 2\%$ in patients with syndrome X compared with $35 \pm 3\%$ in matched control subjects at 90 min ($p < 0.001$). Although plasma endothelin-1 concentrations were not significantly higher in patients with syndrome X (4.8 vs. 4.0 pg/ml, $p = 0.17$), the vasoconstriction caused by endothelin-1 infusion correlated inversely with plasma endothelin-1 concentrations ($r = -0.51$, $p = 0.04$).

Conclusions. Patients with syndrome X had normal basal and stimulated nitric oxide activity and basal endogenous ET_A receptor-mediated vascular tone. However, despite otherwise normal vascular function, there was reduced responsiveness to exogenous endothelin-1, possibly reflecting overactivity of this system and ET_A receptor downregulation.

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Over 100 years ago, Sir William Osler described a condition of "hysterical angina" or "pseudoangina" in patients who had typical anginal pain but were found to have normal coronary arteries after death (1). With the emergence of electrocardiography and coronary angiography, this condition became known as syndrome X (2). The precise definition of this syndrome varies among centers and clinicians. However, a relatively homogeneous group of patients can be defined on the basis of typical anginal chest pain, a positive exercise tolerance test and a normal coronary angiogram (3,4).

Results from several major studies suggest that within syndrome X there is a substantial subgroup of patients with abnormal vascular responses. Various investigators have doc-

umented impaired coronary vascular reserve with abnormal responses to vasodilators (5-8) and to atrial pacing (9,10). Some investigators (11) have postulated that in this subgroup of patients with syndrome X, the chest pain is a consequence of increased coronary resistance vessel tone leading to ischemic pain—so-called "microvascular angina"—and that this may be due to endothelial dysfunction (6,8,12). Evidence in favor of an ischemic origin of the chest pain in syndrome X arises from histologic abnormalities seen in myocardial biopsies (13), the reduction of myocardial blood flow (by positron emission tomography and thallium scanning) (7,13,14) and the identification of elevated coronary sinus lactate concentrations (10). Moreover, the vascular abnormalities of syndrome X are not confined to the coronary circulation because impaired reactive hyperemia to forearm ischemia (12) and structural alterations in the small arteries of the skin and subcutaneous tissue (15) have been detected. Therefore, there may be a generalized dysfunction of vascular or endothelial function, as opposed to a local coronary abnormality, in syndrome X.

Both the nitric oxide and endothelin systems contribute to the maintenance of basal peripheral vascular tone in humans (16,17). The aim of the current study was to assess the contribution and action of nitric oxide and endothelin-1 in

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Abbreviations and Acronyms

ANOVA	= analysis of variance
ECG	= electrocardiogram
ET _A receptor	= endothelin type A receptor
ET _B receptor	= endothelin type B receptor
L-NMMA	= L-N ^G -monomethyl arginine
vWF	= von Willebrand factor

peripheral resistance vessels of patients with syndrome X. The peripheral vascular actions of endogenous and exogenous nitric oxide and endothelin-1 were determined using the nitric oxide synthase inhibitor, L-N^G-monomethyl arginine (L-NMMA), the nitric oxide donor, sodium nitroprusside, the endothelin type A (ET_A) receptor antagonist, BQ-123, and endothelin-1 peptide. In addition, endothelial cell function was assessed using the endothelium-dependent, nitric oxide-generating vasodilators, substance P and acetylcholine (18).

Methods

Patients. Patients with syndrome X were recruited according to the criteria in Table 1 and were matched by age and gender with healthy control subjects. The patients had undergone coronary angiography and exercise treadmill tests within 12 months of the study and were considered to have normal smooth coronary arteries on angiography by two independent cardiologists. All subjects attended a screening visit and received a clinical examination, rest electrocardiogram (ECG), echocardiogram and oral glucose tolerance test. Control subjects did not have a history of chest pain or clinically significant disease and had a normal rest ECG and echocardiogram.

Studies were undertaken with the approval of the Lothian Research Ethics Committee and were in accordance with the Declaration of Helsinki. Each subject gave written informed consent before entry into the study.

Assays. Plasma endothelin-1 (Peninsula Laboratories Europe Ltd., St. Helens, United Kingdom) and big endothelin-1 (Peninsula Laboratories Europe Ltd.) were determined by radioimmunoassay (19); von Willebrand factor (vWF) antigen (Dako A/S, Glostrup, Denmark) and insulin (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) by an enzyme-linked immunosorbent assay; and nonesterified fatty acid (Wako, Neuss, Germany), triglyceride (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) and cholesterol (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) by an enzymatic colorimetric method. Low density lipoprotein cholesterol was determined by the method of Friedewald et al. (20).

Study design. Blood flow was measured in both forearms by venous occlusion plethysmography using mercury-in-Silastic strain gauges applied to the widest part of the forearm, as described previously (17,21). Blood pressure was monitored in the noninfused arm at intervals throughout each study using a semiautomated, noninvasive oscillometric sphygmomanometer (22) (Takeda UA 751, Takeda Medical Inc, Tokyo, Japan). The brachial artery of the nondominant arm was cannulated with a 27-standard wire gauge steel needle (Cooper's Needle Works Ltd., Birmingham, United Kingdom) under 1% lidocaine (xylocaine; Astra Pharmaceuticals Ltd., Kings Langley, United Kingdom) local anesthesia. The total rate of intraarterial infusions was maintained constant throughout all studies at 1 ml/min. Substance P (Clinalfa AG, Läufelfingen, Switzerland), sodium nitroprusside (Nipride; Roche, Welwyn Garden City, United Kingdom), acetylcholine (Miochol; Iolab, Brack-

Table 1. Inclusion and Exclusion Criteria for Study

Inclusion Criteria	Exclusion Criteria
Age between 21 and 75 years	Hypertension (systolic blood pressure >160 mm Hg or diastolic blood pressure >90 mm Hg)
Typical anginal chest pain	Diabetes mellitus
≥2-mm horizontal or downsloping ST segment depression (60 ms after the J point) on exercise treadmill testing using the Bruce protocol	Echocardiography
Angiographically normal smooth coronary arteries	Left ventricular hypertrophy (>1.3-mm thickness of posterior wall of left ventricle)
	Regional wall motion abnormalities
	Valvular heart disease (trivial mitral incompetence was permitted)
	Impaired ventricular function
	Electrocardiography
	Left ventricular hypertrophy (>35 mm from summation of amplitude of S wave in lead V ₁ and R wave in lead V ₅ or V ₆)
	Left bundle branch block
	Pathologic Q waves or T wave inversion
	Any evidence of coronary artery atherosclerosis
	Previous myocardial infarction
	History of dyspepsia, peripheral vascular disease or cerebrovascular disease
	Other significant cardiac disease

Table 2. Characteristics of Study Group

	All Subjects		Nitric Oxide Studies		Endothelin Studies	
	Pts With Syndrome X	Control Subjects	Pts With Syndrome X	Control Subjects	Pts With Syndrome X	Control Subjects
Age (years)	56 ± 1	55 ± 3	56 ± 2	54 ± 3	54 ± 3	55 ± 4
Gender (M/F)	4/11	4/7	4/6	4/6	3/7	3/7
Body mass index (kg/m ²)	28 ± 1	25 ± 1*	28 ± 2	25 ± 1*	28 ± 2	25 ± 1*
Blood pressure (mm Hg)						
Systolic	138 ± 4	127 ± 3	139 ± 5	127 ± 3	137 ± 4	128 ± 3
Diastolic	78 ± 1	77 ± 2	79 ± 2	77 ± 2	79 ± 2	78 ± 2
Heart rate (beats/min)	71 ± 1	70 ± 1	70 ± 1	70 ± 1	70 ± 1	70 ± 1
ST segment depression during treadmill exercise testing (mm)	2.7 ± 0.2	—	2.6 ± 0.2	—	2.7 ± 0.1	—
Plasma endothelin-1 (pg/ml)	4.8 ± 0.5	4.0 ± 0.2	4.5 ± 0.6	4.0 ± 0.2	4.8 ± 0.5	4.0 ± 0.3
Plasma big endothelin-1 (pg/ml)	20 ± 2	18 ± 2	17 ± 0.7	18 ± 1.7	20 ± 1.5	19 ± 1.9
Plasma vWF (IU/ml)	1.17 ± 0.12	0.98 ± 0.05	1.13 ± 0.16	0.97 ± 0.05	1.3 ± 0.2	0.98 ± 0.05
Total cholesterol (mg/dl)	258 ± 15	268 ± 23	260 ± 21	257 ± 23	258 ± 23	276 ± 24
LDL cholesterol (mg/dl)	174 ± 13	197 ± 22	175 ± 18	186 ± 22	178 ± 19	202 ± 24
HDL cholesterol (mg/dl)	55 ± 4	47 ± 5	58 ± 5	47 ± 5	50 ± 5	49 ± 5
Triglycerides (mg/dl)	141 ± 15	120 ± 22	134 ± 18	121 ± 24	146 ± 19	124 ± 25
Oral glucose tolerance						
Fasting glucose (mmol/liter)	4.9 ± 0.2	4.9 ± 0.2	5.0 ± 0.2	4.9 ± 0.1	5.0 ± 0.1	4.9 ± 0.2
2-h glucose (mmol/liter)	5.1 ± 0.4	5.2 ± 0.4	5.0 ± 0.4	4.9 ± 0.4	5.2 ± 0.5	5.4 ± 0.4
Fasting insulin (IU/ml)	8.9 ± 1.5	10.9 ± 2.7	7.2 ± 1.3	9.7 ± 2.7	9.9 ± 2.1	11.6 ± 2.9
2-h insulin (IU/ml)	45.3 ± 14.5	25.8 ± 5.9	36.5 ± 15	22.6 ± 5.6	59.0 ± 22.5	28.0 ± 6.1
Fasting NEFA (mEq/liter)	584 ± 71	572 ± 76	541 ± 60	550 ± 81	626 ± 108	604 ± 77
2-h NEFA (mEq/liter)	26 ± 12	33 ± 13	29 ± 17	36 ± 14	32 ± 18	36 ± 14
Thyroid stimulating hormone (mU/liter)	2.0 ± 0.6	1.3 ± 0.3	2.0 ± 0.6	1.4 ± 0.3	2.5 ± 0.8	1.2 ± 0.2
Thyroxine (pmol/liter)	15.2 ± 0.9	15.3 ± 0.8	15.1 ± 1.0	15.3 ± 0.8	14.9 ± 1.0	15.6 ± 0.8
Urea (mmol/liter)	5.8 ± 0.3	5.2 ± 0.3	5.8 ± 0.3	5.2 ± 0.3	5.8 ± 0.4	5.1 ± 0.4

*p = 0.02 (unpaired *t* test). Data are presented as mean value ± SD. F = female; HDL = high density lipoprotein; LDL = low density lipoprotein; M = male; NEFA = nonesterified fatty acids; Pts = patients; vWF = von Willebrand factor.

nell, United Kingdom), L-NMMA (Clinalfa AG), endothelin-1 (Clinalfa AG) and BQ-123 (American Peptide Company) were administered after dissolution in 0.9% saline (Baxter Healthcare Ltd., Thetford, United Kingdom). Before the subjects underwent each of the studies, aspirin was discontinued for 10 days and vasoactive or nonsteroidal anti-inflammatory drugs for at least 5 half-lives. All subjects abstained from alcohol for 24 h and from food and caffeinated drinks for at least 5 h before each study. Subjects rested recumbent in a quiet, temperature-controlled room maintained at 23.5 to 24.5°C. Before participating in one of the following protocols, saline was infused for the first 30 min to allow time for equilibration, with forearm blood flow measured every 10 min and the final measurement taken as basal blood flow.

Nitric oxide system. In 10 patients with syndrome X and 10 age- and gender-matched control subjects, intrabrachial substance P was administered at 1, 2 and 4 pmol/min (18,23); sodium nitroprusside at 5, 15 and 30 nmol/min (23); and acetylcholine at 27.5, 55 and 110 nmol/min (16,18,23) for 6 min at each dose. Administration of the three agents was separated by 20-min saline infusions and given in random order. Finally, after a further 20-min saline infusion, L-NMMA was administered at 4 μmol/min (16,18) for 10 min. Forearm blood flow measurements were made for the last 3 min of each infusion period.

Endothelin system. In 10 patients with syndrome X and 10 age- and gender-matched control subjects, intrabrachial BQ-123 was administered at 10 nmol/min (17) for 90 min. On a separate study day, at least 1 month later, endothelin-1 was administered at 5 pmol/min (17) for 90 min. Forearm blood flow measurements were made for 3 min every 6 min.

All subjects were not able to attend both the nitric oxide and endothelin studies; five patients and nine control subjects were common to both phases.

Data analysis and statistics. Study group size, based on reproducibility data derived from forearm resistance vessel responses to endothelin-1, gave a 90% power to detect a 24% difference in blood flow responses at a significance level of 5%. Data were examined, as appropriate, by two-way analysis of variance (ANOVA) with repeated measures, the two-tailed unpaired Student *t* test and regression analysis using Excel version 5.0 (Microsoft). All results are expressed as the mean value ± SEM. Statistical significance was set at the 5% level.

Results

Subject characteristics. Patients with syndrome X were well matched with control subjects for age, gender and serum lipid and thyroid profiles (Table 2). The rest ECG was normal in both patients and control subjects, with no baseline ST

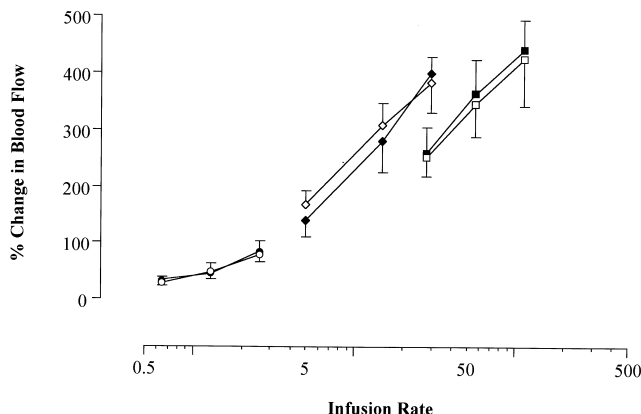


Figure 1. Responses in forearm blood flow to incremental doses of substance P (circles [pmol/min]), acetylcholine (squares [nmol/min]) and sodium nitroprusside (triangles [nmol/min]) in patients with syndrome X (solid symbols) and control subjects (open symbols). There were no significant differences between patients and control subjects.

segment changes. Body mass index was significantly higher in the patients with syndrome X ($p = 0.02$), with a trend ($0.10 > p > 0.05$) toward higher systolic blood pressures and 2-h plasma insulin concentrations. There were no significant differences in other hemodynamic or metabolic variables. There were no significant differences in mean plasma vWF ($p = 0.16$), endothelin-1 ($p = 0.17$) or big endothelin-1 concentrations ($p = 0.58$).

Nitric oxide system. There were no significant differences in length (25.4 ± 0.7 vs. 25.2 ± 0.7 cm) or basal blood flow (3.8 ± 0.4 [range 2.9 to 9.2] vs. 3.3 ± 0.5 [range 1.0 to 6.3] ml/100 ml per min) of the infused forearm in patients with syndrome X versus control subjects (24). There were no significant changes in blood pressure, heart rate or blood flow in the noninfused forearm during the course of the studies.

Substance P, acetylcholine and sodium nitroprusside all caused dose-dependent vasodilation in the infused forearm ($p < 0.001$ for all) (Fig. 1). L-NMMA caused a $36.7 \pm 3.4\%$ ($p < 0.001$) and $38.4 \pm 3.1\%$ ($p = 0.007$) reduction in forearm blood flow in patients with syndrome X and control subjects, respectively. There were no significant differences in the blood flow responses to substance P, acetylcholine, sodium nitroprusside or L-NMMA between patients and control subjects.

Endothelin system. There were no significant differences in basal blood flow of the infused forearm on the endothelin-1 (3.5 ± 0.8 [range 2.3 to 6.2] vs. 4.2 ± 0.5 [range 1.8 to 10.2] ml/100 ml per min) and BQ-123 (3.2 ± 0.9 [range 1.7 to 6.4] vs. 4.1 ± 0.5 [range 1.0 to 10.0] ml/100 ml per min) study days in patients with syndrome X versus control subjects. There were no significant changes in blood pressure, heart rate or blood flow in the noninfused forearm during the course of the BQ-123 and endothelin-1 study days.

BQ-123 caused a progressive vasodilation ($p < 0.001$ by ANOVA for both groups), which appeared to reach a maximum by 72 min (Fig. 2). At 90 min, forearm blood flow was increased by $39 \pm 6\%$ in the patients with syndrome X and

$37 \pm 9\%$ in the control subjects. There was no significant difference between patients and control subjects.

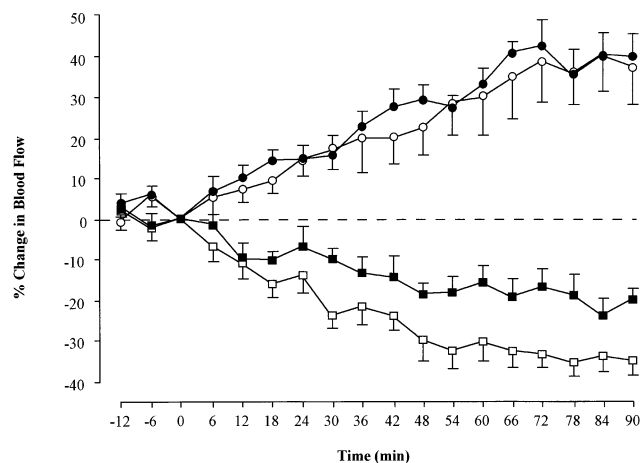
Endothelin-1 caused a progressive vasoconstriction ($p < 0.001$ by ANOVA for both groups), which appeared to reach a maximum by 66 min (Fig. 2). The reduction in forearm blood flow was significantly ($p < 0.001$ by two-way ANOVA) less in the patients with syndrome X ($20 \pm 2\%$ [range 9% to 29%] at 90 min) than in the control subjects ($35 \pm 3\%$ [range 25% to 53%]) at 90 min. Using data from all subjects, the degree of vasoconstriction produced by endothelin-1 infusion was inversely correlated with plasma endothelin-1 concentrations ($r = -0.51$, $p = 0.04$), but the correlation with mean arterial pressure was not significant ($r = 0.19$, $p = 0.41$).

Discussion

A number of previous studies have used broader criteria for defining syndrome X, including patients with ≥ 1 mm ST segment depression (8,25-27) and those with minor, "hemodynamically nonsignificant" atherosclerotic plaques in the coronary arteries (27,28). In an attempt to define a more precise group of patients with syndrome X, we employed criteria of ≥ 2 mm ST segment depression on exercise testing in combination with typical anginal chest pain and normal smooth coronary arteries on angiography. Patients with other potential causes of chest pain or with coexisting conditions associated with microangiopathy were excluded. In agreement with previous studies (26,29), our patients with syndrome X tended to be insulin-resistant, although they also had a larger body mass index and tended to have a higher systolic blood pressure.

Syndrome X and endothelin-1. For the first time, to our knowledge, we report that patients with syndrome X, as compared with control subjects, have a reduced responsiveness of peripheral resistance vessels to endothelin-1 despite normal vasodilation to the ET_A receptor antagonist BQ-123. In an earlier study with a larger number of patients ($n = 40$), Kaski

Figure 2. Responses in forearm blood flow to 90-min infusions of BQ-123 (circles) and endothelin-1 (squares) in patients with syndrome X (solid symbols) and control subjects (open symbols). $p < 0.001$ between patients and control subjects for endothelin-1 response.



et al. (25) reported a significant elevation in plasma endothelin-1 concentrations (3.8 ± 1.3 vs. 2.9 ± 0.7 pg/ml) in patients versus control subjects. We did not demonstrate a significant elevation in plasma endothelin-1 or big endothelin-1 concentrations in our patients with syndrome X, although the magnitude was similar to the previous study and probably reflects the fact that the study was not powered to detect such a difference. However, interestingly, we did find an inverse correlation between endothelin-1-induced vasoconstriction and circulating plasma endothelin-1 concentrations. This suggests that in the presence of higher endothelin-1 concentrations, possibly related to increased endothelin generation, there may be ET_A receptor downregulation such that the overall contribution of ET_A receptor-mediated vascular tone remains unchanged. Although ET_A receptor downregulation may explain the reduction in vasoconstriction to exogenous endothelin-1, endothelin type B (ET_B) receptor function may also be important and merits further investigation. Indeed, abnormalities of ET_B receptor function have been demonstrated in the coronary vessels of an animal model of heart failure (30), in the peripheral resistance vessels of patients with chronic heart failure (31) and in human atherosclerotic vessels (32).

We have recently shown that the majority of the vasodilation seen with selective ET_A receptor antagonism results from nitric oxide release (33). Given our findings of normal nitric oxide-mediated responses in syndrome X, it may not be surprising that the BQ-123 responses were similar in the two groups. Thus, detecting a reduction or augmentation of endogenous endothelin-1-mediated vasoconstriction may be obscured by the nitric oxide release seen with BQ-123.

Syndrome X and nitric oxide. Despite evidence in support of a nitric oxide-mediated endothelial dysfunction involving the coronary resistance vessels of patients with syndrome X (6,8,28,34), we were unable to detect a significant abnormality affecting normal basal and stimulated release of, and sensitivity to, nitric oxide in the resistance vessels of the forearm circulation in vivo. This is in agreement with the findings of normal endothelium-dependent and -independent nitric oxide-mediated vasorelaxation in structurally abnormal peripheral resistance arteries of patients with syndrome X studied ex vivo (15). However, in a post hoc analysis, we did find that plasma concentrations of vWF were above the normal range (0.42 to 1.22 IU/ml) in six patients with syndrome X, but in none of the control subjects ($p < 0.01$ by chi-square test). Given our findings and those of Kaski et al. (25), it would appear that patients with syndrome X may have a generalized endothelial dysfunction that does not universally affect the nitric oxide system.

Study limitations. The failure to detect differential responses to endothelium-dependent nitric oxide-mediated peripheral vascular responses may reflect the relative hypercholesterolemia of the study groups. Both the patient and control groups had relatively high mean serum cholesterol concentrations, although this is consistent with the average prevailing serum cholesterol concentrations in the Scottish population

(35). This may have conferred some degree of endothelial dysfunction on the subjects (36) and obscured the contribution of further dysfunction. In addition, the slow onset and offset of action of endothelin agonists and antagonists mean that only one dose of each agent can be administered on each study day. Thus, a full dose-response relation cannot easily be determined. Moreover, further characterization of responses mediated by the ET_B receptors is now needed to further clarify this response. There also remains the possibility that the observed differences are related to the disparity in body mass index between the two groups.

Conclusions. Our study suggests that despite normal overall peripheral resistance vessel function, there appears to be a reduced responsiveness to endothelin-1 in patients with syndrome X, consistent with increased endothelin-1 production and ET_A receptor downregulation. Further studies are now required to fully characterize the role of the endothelin system in both the peripheral and coronary circulations in this condition.

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